10/825921

Cofc

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450 Alexandria, VA 22313 on May 15, 2007.

Frank C. Eisenschenk

Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. UF-174D4
Patent No. 7,192,730

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	David Michael Young
Issued	:	March 20, 2007
Patent No.	:	7,192,730
For	:	Thermostable Proteolytic Enzymes and Uses Thereof in Peptide and Protein Synthesis

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Column 9, line 16:	Page 14, line 18:
"ofthis band"	--of this band--

Column 9, line 55:	Page 15, line 15:
"In c vs r^{2}"	--ln c *vs* r^{2}--.

A true and correct copy of pages 14 and 15 of the specification as filed which support Applicant's assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,

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FCE/jps

Attachments: Copy of pages 14 and 15 of the specification

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d. The pooled fractions from (c) are concentrated by membrane filtration and applied to a 2.6 x 60 cm column of Superdex-200 equilibrated with 0.1 M tris-HCl, 0.1 NaCl, pH 7.5.

Throughout the above ion-exchange steps (a-c), the chromatographic profiles reveal 2 prominent protein peaks that display BAPNA anilidase activity, together with other BAPNA-positive peaks in much lower amounts that are successively eliminated with each column step. The last step (gel filtration) yields 2 well-separated protein fractions that represent approximately 80% and 15% of the anilidase activity present in the original cell sonicate. The most abundant of these 2 proteins is the one used for all of the studies described below. It emerges from the Superdex-200 column with an apparent molecular weight of about 110,000 as judged from its partition coefficient determined with standard gel filtration molecular weight marker proteins. Polyacrylamide gel electrophoresis (SDS-PAGE) yields a single sharp band under reducing conditions. The estimated molecular weight of this band is approximately 81,000. The yield is approximately 1 mg of pure protein from 100 g wet cells.

Example 2 – Extinction Coefficient, Apparent Partial Specific Volume, and Molecular Weight of Serine Protease of the Subject Invention

The protein was hydrolyzed (constant boiling HCl) for 18, 22, 24 and 26 hours. From absorbance measurements (280 nm) and the methods of Edelhoch (Edelhoch, H. "Spectroscopic Determination of Tryptophan and Tyrosine in Proteins." *Biochemistry* 6:1948-1954, 1967), the extinction coefficient was calculated to be 1.31 ml $mg^{-1}cm^{-1}$.

Sedimentation equilibrium measurements utilized a Beckman Model E ultracentrifuge equipped with a split-beam scanner and multiplexer for visualization of two centrifuge cells during the same run. The high speed method of Yphantis (Yphantis, D.A. "equilibrium Ultracentrifugation of Dilute Solutions," *Biochemistry* **3**:294-303, 1964) was employed together with the methods of Edelstein and Schachman for simultaneous measurement of the partial specific volume (Edelstein, S.J. and Schachman, H.K. "The

Simultaneous Determination of Partial Specific Volumes and Molecular Weights with Microgram Quantities." *J. Biol. Chem.* 242:306-311, 1967). One cell contained protein dialyzed thoroughly against 0.1 M tris-HC1, pH 7.5, in H_2O and the second cell contained the enzyme in the same buffer with 99% D_2O as solvent (densities of the buffer solutions were measured pycnometrically). Centrifugation (20,000 RPM, 23.5EC) yielded a molecular weight of approximately 81,500 and an apparent partial specific volume (Casassa, E.F. and Eisenberg, H. "Thermodynamic Analysis of Multicomponent Solutions." *Adv. Prot. Chem.* 19:287-393, 1964) of 0.789 ml/g. This is a surprisingly high value for the specific volume of a protein and it implies a larger than expected Stokes radius, which may explain why the protein emerges earlier upon gel filtration than would be anticipated for a protein of molecular weight of 81,500. Plots of ln c *vs* r^2 were strictly linear–a feature that indicates size homogeneity. The close similarity of the molecular weight to that obtained by SDS-PAGE indicates that the protein has a single polypeptide chain structure.

Example 3 – Stability of Enzymic Activity at High Temperature

For all kinetic experiments at high temperatures, sodium phosphate (0.025 M) was used as a buffer. The temperature coefficient of this buffer is so small that slight changes in pH with temperature do not significantly affect the kinetic data.

To assess stability of the enzyme at high temperature, a solution of the protein in the above buffer, pH 7.0, was incubated at 82.0 +/- .05°C. Aliquots were removed at hourly intervals up to 8 hr, and initial velocities were measured (BAPNA as substrate, Varian 2290 recording spectrophotometer) at 25.0° +/- .05°C (Erlanger, B.F., Kokowski, N. and Cohen, W. "The Preparation and Properties of Two New Chromogenic Substrates of Trypsin." *Arch. Biochem. Biophys.* 95:271-278, 1961). No decrease in enzyme activity was observed over this time period.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,192,730

APPLICATION NO.: 10/825,921

DATED : March 20, 2007

INVENTOR : David Michael Young

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 9,
Line 16, "ofthis band" should read --of this band--.

Column 9,
Line 55, "In c vs r^2" should read --ln c *vs* r^2--.

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

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